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Research Article

Stable isotope analysis reveals diet niche partitioning between native species and the invasive black bullhead (*Ameiurus melas* Rafinesque, 1820)

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Abstract

The introduction and spread of alien fish species pose a major threat to native communities and ecosystem functioning in freshwaters. Black bullhead is one of the most successful invaders in European waters with several detrimental effects on native biota and ecosystems. In this study, we used stable isotope analysis to compare the body size and season-dependent diet, trophic position, isotopic niche size, and niche overlap of the invasive black bullhead with two native fish species (roach and European perch) in Lake Balaton, Hungary. We found that black bullhead could be characterized by invertivore-piscivorous feeding habit with a high rate of fish consumption. The rate of fish predation by invasive black bullhead increased with body size, while no seasonal differences were observed in fish consumption. Contrary to our hypothesis, little evidence of actual feeding competition was found between black bullhead and native fishes. Our results suggest that the studied species assimilate distinct energy resources in different proportions leading to a substantial amount of niche partitioning among them. We conclude that black bullhead may represent a threat for native, small-sized fishes primarily through predation and recommend urgent management actions (e.g. selective removal of the species) to minimize its adverse impacts on native communities.

Key words: Biotic interactions, fish, non-native, piscivory, predation, trophic position

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Introduction

The occurrence and spread of invasive species are leading drivers of biodiversity loss and pose major threats to ecosystem integrity. Adverse impacts of invasive species can be various and may occur at multiple ecological levels from single prey-predator relationships and competition (Bergstrom and Mensinger 2009; Seiler and Keeley 2009; Remon et al. 2016; Somogyi et al. 2023) to shifts in ecosystem functioning (Dietrich et al. 2006; Crowl et al. 2008; Linders et al. 2019). Freshwaters, and especially lakes, are often subject to high rates of species introductions, despite these ecosystems being known to be particularly vulnerable and susceptible to human activities and biological invasions (Gozlan et al. 2010; Mandrak and Cudmore 2010; Miró and Ventura 2013). Lakes cover only a limited area worldwide

but support diverse species, including endemic taxa, and contribute substantially to ecosystem services (Dudgeon et al. 2006; Heino et al. 2021). However, habitat degradation, climate change and biological invasions have led to a considerable decline in species richness in these ecosystems occurring at a faster rate than in terrestrial and marine counterparts (Johnson et al. 2008; Strayer and Dudgeon 2010).

Several non-native fish species have been introduced into European freshwaters during the past decades mainly to support for commercial purposes, sport fishing or the ornamental market (Holčík 1991; Gozlan 2008). These species adversely affect the structure and functioning of native fish assemblages (Cucherousset and Olden 2011; van der Veer and Nentwig 2015) by altering natural aquatic food webs and competing for food resources with native species (Khan and Panikkar 2009; Britton et al. 2010; Bezerra et al. 2018). Black bullhead (*Ameiurus melas* Rafinesque, 1820) is one of the most successful invaders in European waters with detrimental ecological and socio-economic impacts (Leunda et al. 2008; Copp et al. 2016; Ferincz et al. 2016). The species was introduced to Europe from North America in the late 19th and early 20th centuries. Its fast spread was supported by physiological and life-history traits such as high fecundity, parental care, omnivory and tolerance to water pollution and low oxygen concentration (Ribeiro et al. 2008; Novomeská and Kováč 2009).

Black bullhead is an effective competitor and predator of native fish species. For example, Jaćimović et al. (2021) highlighted the opportunistic generalist feeding of the species with fish as a main prey item in its diet, followed by aquatic invertebrates. Similarly, Leunda et al. (2008) and Ruiz-Navarro et al. (2015) indicated that the species consumed co-occurring fish beside plant material and invertebrates affecting negatively the native ichthyofauna through both direct predation and competition. Additionally, Kreutzenberger et al. (2008) found the reduced predatory efficiency of pike (*Esox lucius* Linnaeus, 1758) in the presence of black bullhead and emphasized the potential adverse impact of this invasive species on native communities. However, there is a dearth of information on the position and specific role of black bullhead in the food web in its introduced range. While general knowledge suggests that black bullhead is an omnivorous species, there is less information about the seasonal and body size-dependent variation of its diet. In addition, the lack of detailed information on diet-based niche overlap between black bullhead and native fishes may hinder the planning of effective management strategies to control the negative impacts of this invasive species.

Stable isotope analysis (SIA) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) is a powerful method to examine the general feeding habits of fish (Vander Zanden et al. 1999; Britton et al. 2010; Dominguez Almela et al. 2021). Unlike gut-content analysis, which provides information about the recently consumed preys and is biased towards the underestimation of the importance of easily digestible food items, SIA provides longer-term and time-mediated information about a species' diet and trophic niche (Balzani et al. 2020; McCue et al. 2020). In fact, stable nitrogen isotope ratio is an appropriate measure of consumer trophic position as it becomes enriched by 3–4‰ between prey and predator tissues (Cabana and Rasmussen 1994; Vander Zanden et al. 1999). Stable carbon isotope ratios exhibit little or no trophic level enrichment and are similar between the prey and the consumer providing a useful indicator of nutrient and energy source (Vander Zanden et al. 1999; Cucherousset et al. 2012). Stable isotope analysis thus can allow inferences regarding competition and niche partitioning among species and has been shown

to be a useful method in determining the ecological impacts of alien fishes (Post 2002; Cucherousset et al. 2012).

The objective of this study was to compare the diet and the isotopic niche size and niche overlap of the invasive black bullhead and two native fish species. Since previous gut content analysis-based studies suggested that black bullhead is typically omnivorous with a dominance of invertebrate and fish preys in its diet (Leunda et al. 2008; Jaćimović et al. 2021), we chose another omnivorous cyprinid fish, the roach (*Rutilus rutilus* Linnaeus, 1758) and an invertivore-piscivorous percid fish, the European perch (*Perca fluviatilis* Linnaeus, 1758, perch hereafter) to infer potential competition between black bullhead and the native fish species in Lake Balaton, Hungary. We were especially interested to explore the seasonal and/or body size-dependent pattern in niche size and niche overlap among the species. Our predictions were as follows: (i) black bullhead will exhibit omnivorous feeding (i.e., broad niche size) with a preference to animal prey (ii) it will occupy an intermediate trophic position between perch and roach, and therefore acts as a strong competitor of both native fishes, (iii) the rate of predation on fish by black bullhead will increase with body size, and (iv) the adverse effects of black bullhead will be permanent throughout the year, that is the level of potential competition (niche overlap) with native fishes and the rate of fish in diet will not show seasonal differences.

Methods

Study area

Lake Balaton is the largest lake (surface area: 593 km^2; mean depth: 3.2 m) in Central Europe situated at 46°42'–47°04'N, 17°15'–18°10'E and 104.8 m above sea level. The lake is typically turbid and mesotrophic (Istvánovics et al. 2007). About 40% of the littoral zone is covered by common reed [*Phragmites australis* (Cav.) Trin. ex Steud.] (Specziár et al. 2013), but the rest of the shoreline is anthropogenically modified (mainly riprap sections, beaches, and harbours). Recently, 34 fish species occur in the lake, from which 9 species are invasive. Beside black bullhead, the following invasive fishes occur in Lake Balaton: Asian carp species and their hybrids (silver carp, *Hypophthalmichthys molitrix* Valenciennes, 1844 and bighead carp, *Hypophthalmichthys nobilis* Richardson, 1845) pumpkinseed (*Lepomis gibbosus* Linnaeus, 1758), monkey goby (*Neogobius fluviatilis* Pallas, 1814), tubenose goby (*Proterorhinus semilunaris* Heckel, 1837), Prussian carp (*Carassius gibelio* Bloch, 1782), topmouth gudgeon (*Pseudorasbora parva* Temminck & Schlegel, 1846), grass carp (*Ctenopharyngodon idella* Valenciennes, 1844). Detailed information on the limnology and fish fauna of the lake can be found in Bíró (1997), Istvánovics et al. (2007) and Takács et al (2017).

Studied fish and collection of organisms

Fish were collected by fyke netting and electrofishing in Lake Balaton during three sampling periods in 2022: (1) spring (from 26 May to 28 May), (2) summer (from 15 August to 25 August), and (3) autumn (from 29 October to 3 November). Fyke nets were inspected and emptied every day. The net frame had a length of 80 cm with an easily expanding 15 cm throat size. Mesh size of the net was 8 mm. Electrofishing was performed using a backpack electrofishing gear (IG200/2B, PDC,

50–100 Hz, 350–650 V, max. 10 kW; Hans Grassl GmbH, Germany) from a rubber boat in the littoral zone of the lake during night-time. Two size groups of each species were collected in each season. The smaller size group included 1+ year old individuals with a size of 8–11 cm standard body length (SL), while the larger size group contained 3+ years old individuals with a size of 17–23 cm SL. Altogether, 15 individuals were collected of each species in each season and size group. Collected individuals were immediately transported into the Balaton Limnological Research Institute where they were stored frozen (-20 °C) until the laboratory processing (within 1–2 weeks).

The most common potential food items in Lake Balaton suggested by Specziár (2010) were also collected. These were benthic chironomid larvae (*Chironomus balatonicus* Dévai, Wülker & Scholl, 1983), mussel (*Dreissena bugensis* Andrusov, 1897), snail (*Theodoxus fluviatilis* Linnaeus, 1758)), crayfish (*Faxonius limosus* Rafinesque, 1817), amphipod (*Dikerogammarus spp.*), benthic filamentous green algae (*Cladophora glomerata* Kützing, 1843) and a mixture of submerged aquatic macrophytes (*Ceratophyllum demersum* Linnaeus, 1753; *Myriophyllum spicatum* Linnaeus, 1753; *Najas marina* Linnaeus, 1753; *Potamogeton perfoliatus* Linnaeus, 1753). Sediment samples were collected using an Ekman grab sampler and chironomid larvae were separated from the sediment by washing the samples through a 0.25 mm mesh sieve. Crayfish individuals were caught by fyke netting with the same type of fyke net used for fishing. Mussels, snails, amphipods, and filamentous green algae were collected by hand from the surface of large rocks. Aquatic macrophytes were also collected by hand and washed with lake water to remove the periphyton and deposited inorganic particles. Small fish were also considered as a potential food item. For this, we used the isotopic signal of a common species in the lake (1+ year old individuals of roach with 5–6 cm SL) collected by electrofishing beside the studied fish. Five samples of each potential food item were collected. Each sample of chironomid larvae, amphipod, mussel and snail represented 10–30 individuals per sample to have sufficient biomass for SIA.

All procedures involving the handling and treatment of animals were in accordance with Hungarian law and the permit for the delivery and use of aquatic animals for scientific purposes (permit reg. no.: VE-I-001/01890-3/2013, valid between 22 August 2013 and 21 August 2023, issued by the Food-Security and Animal Health Directorate, Governmental Office of Veszprém County, Hungary).

Stable isotope analysis

We used $\delta^{13}C$ and $\delta^{15}N$ ratios to examine the diet and provide estimates of niche overlap among black bullhead and native fish species. Dorsal muscle tissues were taken from fish for SIA. All samples were dried to a constant weight at 50 °C before grinding into a fine powder with a mortar and pestle. Stable isotopes were measured with a Thermo Scientific™ EA IsoLink™ IRMS System coupled to a Thermo Finnigan DeltaPLUS XP continuous-flow isotope ratio mass spectrometer. Stable isotope ratios are reported as ‰ with the δ notation as follows:

$$\delta X\,(‰) = [(\text{R sample}/\text{R standard}) - 1] \times 1000$$

where X is ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios. The reference materials used were secondary standards of known relation to the international standards of Vienna

Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen. Each sample was measured at least in three replicates for each stable isotope. Standard deviations of individual $\delta^{15}N$ and $\delta^{13}C$ measurements were ± 0.1 ‰ and ± 0.1 ‰, respectively.

Data analysis

All data analyses were performed in the R environment (R Core Team, 2021). $\delta^{13}C$ and $\delta^{15}N$ ratios were compared among species, seasons and size groups with an aligned rank transform analysis of variance (ART ANOVA) in the package ARTool 0.11.1 (Kay et al. 2021). ART ANOVA is a non-parametric approach to factorial ANOVA which is useful when the data are not normally distributed. A post hoc contrast test (i.e. ART-C) was performed when significant differences were found (Elkin et al. 2021).

For visualization, isotopic niches of species for each season and size group were determined as standard ellipse areas (SEA) using the package SIBER 2.1.6 (Jackson et al. 2011). Standard ellipse area contains approximately 40% of the data and it is the recommended method for comparing core aspects of a population's niche (Layman et al. 2012). Ellipse areas were calculated with small sample size correction (SEAc) (Jackson et al. 2011).

For calculating isotopic niche sizes (SEAc) and niche overlaps between black bullhead and native fish species we used the package nicheROVER 1.1.0 (Lysy et al. 2021). This package uses a probabilistic method to calculate niche size and pairwise niche overlap in a Bayesian framework, accounting for uncertainty due to sample size producing elliptical projections of core isotopic regions (Swanson et al. 2015). To test whether one group's ellipse is smaller or larger than another one, we calculated the probability that its posterior distribution is smaller or larger. We considered differences to be significant if the probability was more than 0.95. For estimating isotopic niche overlap, directional pairwise overlaps (i.e. the probability that an individual of one species fall within the niche of another species in $\delta^{13}C$ and $\delta^{15}N$ bivariate space) were calculated from the posterior distribution of species μ and Σ (Lysy et al. 2021). For exploring the effects of black bullhead on roach and perch, we estimated the proportion of isotopic area of native species overlapped by the isotopic area of black bullhead. Calculations for niche sizes and overlaps were conducted for each season and size group separately, with 10,000 Monte Carlo draws.

We further applied Hotelling T^2 test statistic to examine whether native fishes and black bullhead occupied unique isotopic niches. This test uses a permutation procedure and evaluates the null hypothesis that the Euclidian distance between each pair of centroids does not differ from zero (Turner et al. 2010).

The relative contribution of different food sources was estimated using Bayesian stable isotope mixing models (package MixSIAR 3.1.12) (Stock et al. 2018). The models included the variation of stable isotope values of consumers and its potential food sources as well as variation in the trophic fractionation (i.e. the difference in isotopic composition between a consumer and its diet). We used an uninformative prior. Trophic fractionation values incorporated in the models were 0.4 ± 1.3 ‰ SD for $\delta^{13}C$ and 3.4 ± 1.0 ‰ for $\delta^{15}N$ (Post 2002). Markov chain Monte Carlo sampling was implemented with the following parameters: number of chains = 3; chain length = 100,000; burn in = 50,000; thin = 50 (Stock and Semmens 2016). To test the differences in the rate of fish consumption between the size groups of black bullhead, we calculated the probability that large individuals consumed fish in a higher proportion than small ones from the posterior distribution of MixSIAR

model outputs for each season. Seasonality in black bullhead fish consumption were also explored by comparing the probability that the proportion of fish in the diet is higher in one season than in another one. Similarly to the niche size tests, we considered differences to be significant if the probability was more than 0.95.

For quantifying the relative contribution of littoral and pelagic carbon sources to the diet of each consumer, we carried out two source-one biotracer ($\delta^{13}C$) Bayesian mixing models. For this, we chose one consumer from both the littoral (snail) and the pelagic zone (mussel) of the lake. Snails and mussels are widely used organisms for quantifying the importance of littoral and pelagic carbon sources (Post 2002; Vander Zanden and Vadeboncoeur 2002; Williams and Trexler 2006; e.g. Pingram et al. 2014). Each MixSIAR model was conducted separately for each season and size group of consumers.

Finally, we used a two-baseline model from the package tRophicPosition 0.8.0 (Quezada-Romegialli et al. 2018) to estimate the fish species trophic position in the food web based on the posterior distribution of the model output. Snails and mussels were used as littoral and pelagic sources in the model, respectively. Trophic fractionation values suggested by Post (2002) were used, similarly to MixSIAR models.

Results

ANOVA showed that $\delta^{13}C$ differed significantly among species ($F_{2,252}$ = 116.80, P < 0.0001). Generally, black bullhead was more depleted in $\delta^{13}C$ than perch and roach (contrast tests: P < 0.0001) (Fig. 1), while there was no significant difference between the two native species (contrast test: P = 0.3711). Although, species:season ($F_{4,252}$ = 9.52, P < 0.0001) and species:size group ($F_{2,252}$ = 23.04, P < 0.0001) interactions proved to be also significant, black bullhead $\delta^{13}C$ was the lowest throughout the year and in both size groups (Fig. 1). $\delta^{13}C$ varied also among seasons ($F_{2,252}$ = 3.92, P = 0.0211) as spring samples had significantly higher values than summer samples (contrast test: P = 0.0255). $\delta^{15}N$ differed significantly among species ($F_{2,252}$ = 262.36, P < 0.0001), seasons ($F_{2,252}$ = 21.40, P < 0.0001) and size groups ($F_{1,252}$ = 81.57, P < 0.0001). Although species:season ($F_{4,252}$ = 4.76, P = 0.0010) and species:size group ($F_{2,252}$ = 15.24, P < 0.0001) interactions were also significant, perch had the highest, while roach had the lowest $\delta^{15}N$ throughout the year and in both size groups (contrast test: black bullhead vs. perch: P = 0.0017, black bullhead vs. roach: P < 0.0001, perch vs. roach: P < 0.0001) (Fig. 1). Regarding seasonality, summer samples were generally more enriched while spring samples were more depleted in $\delta^{15}N$ than autumn samples (contrast test: spring vs. summer: P < 0.0001, spring vs. autumn: P = 0.0005, summer vs. autumn: P = 0.0201) except for black bullhead where autumn samples were more enriched than summer and spring samples. Finally, large size group of species were generally more enriched in $\delta^{15}N$ than small size group in each season ($F_{1,252}$ = 81,57, P < 0.0001), except for large roach which was slightly more $\delta^{15}N$ depleted than small one.

Core niche size of black bullhead was significantly (>0.95 probability) smaller in spring for the small and in autumn for the large size group, compared to other seasons (Fig. 1, Appendix 1). Regarding native species, only the small size group of perch showed seasonal changes in niche size; it was largest in autumn and smallest in summer with an intermediate value in spring. Roach had larger niche size than black bullhead in each season and in each size group, although this difference was not significant in spring and summer in the large size group (Fig. 1, Appendix 1). Niche size variability between black bullhead and perch did not show a clear pat-

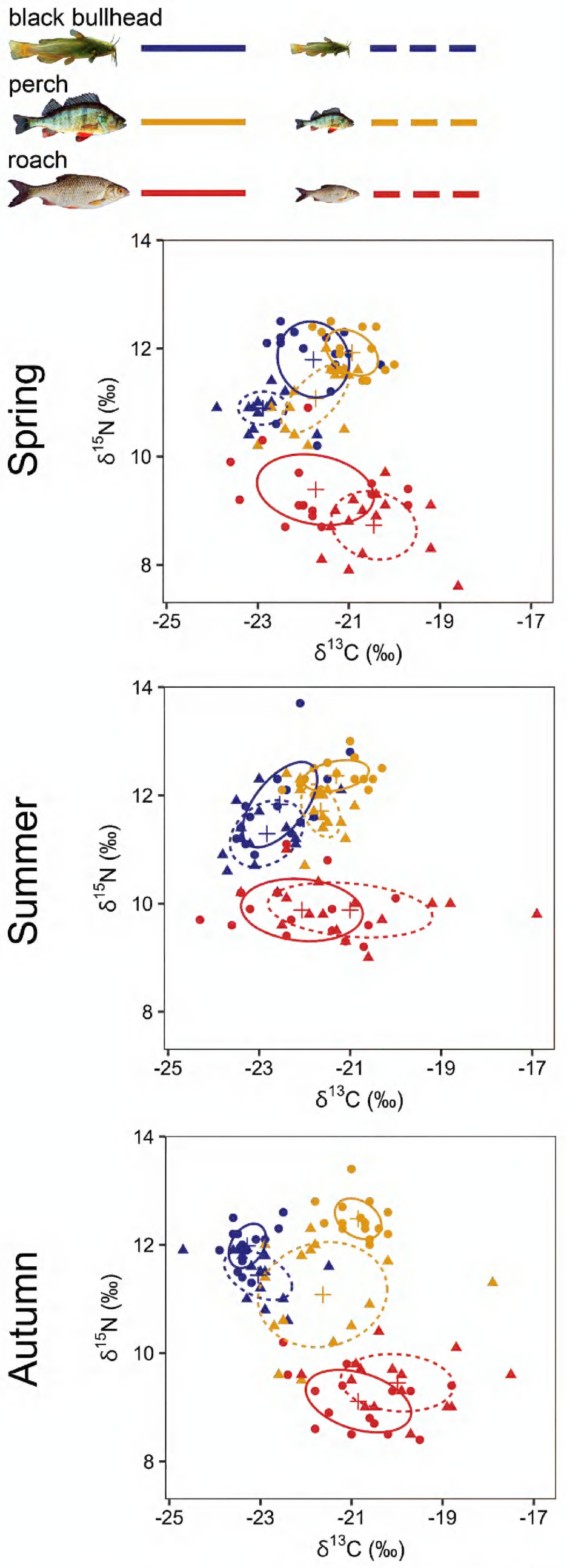

Figure 1. Isotopic niches (SEAc) and their centroids (+) of large and small size groups of black bullhead (*Ameiurus melas*), perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) collected in Lake Balaton (Hungary) in spring (from 26 May to 28 May), summer (from 15 August to 25 August) and autumn (from 29 October to 3 November) of 2022. Axes show the $\delta^{13}C$ and $\delta^{15}N$ values of individuals.

tern. In spring and summer large black bullhead had a significantly larger niche size than large perch, while in autumn, their niche sizes were similar. In small size groups, black bullhead had significantly larger niche size in summer, while significantly smaller niche size in spring and autumn than perch. Overlap between core niches of black bullhead and perch varied among seasons and size groups (Figs 1, 2A, B). Diet niche of both large and small perch was slightly overlapped with large black bullhead showing a decreasing pattern throughout the year with a negligible overlap in autumn (Fig. 2A, B). Diet niche of small perch was also minimally overlapped with small black bullhead in each season (Fig. 2B). Overlap between the core niches of black bullhead and roach was negligible (Fig. 2C, D).

Centroid locations of fish species in the isotopic space varied significantly ($P < 0.05$) in each season, suggesting that species occupy unique trophic niches relative to each other throughout the year. Size groups within species were also separated significantly except for summer black bullhead and summer roach for which Euclidian distance between centroids of their large and small size groups were not significantly different from zero (Fig. 1, Appendix 2).

The most important dietary component of large black bullhead and large perch was fish in each season (Fig. 3). As secondary diet, large individuals of both species

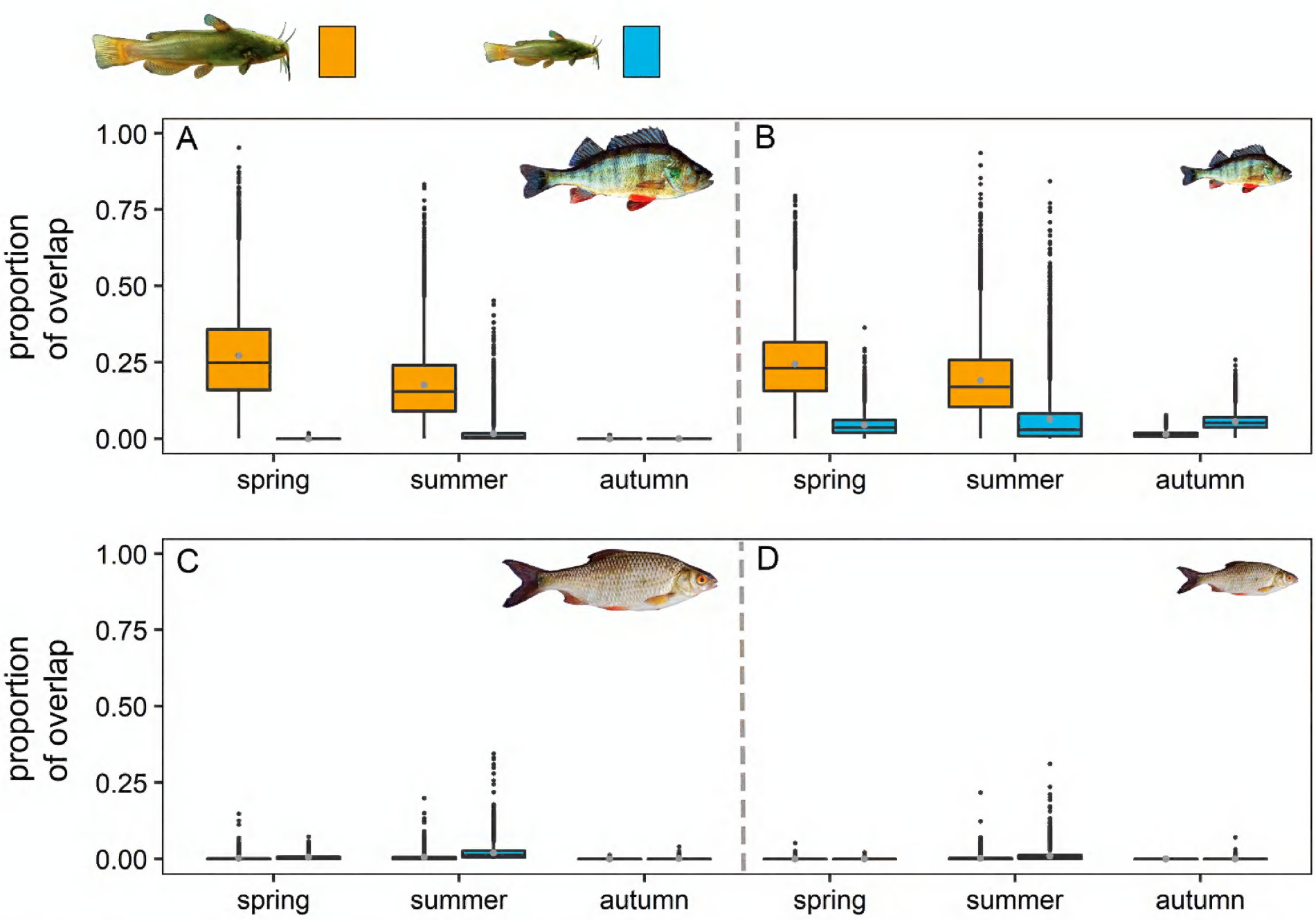

Figure 2. The proportion of isotopic area of large perch (*Perca fluviatilis*) (**A**), small perch (**B**), large roach (*Rutilus rutilus*) (**C**) and small roach (**D**) overlapping with the isotopic area of large and small black bullhead (*Ameiurus melas*) collected in Lake Balaton (Hungary) in spring (from 26 May to 28 May), summer (from 15 August to 25 August) and autumn (from 29 October to 3 November) of 2022. Axes show seasons and the proportion of diet overlap. Whiskers indicate 95% credible intervals with outliers (black dots). Boxplots indicate 25th, 50th and 75th percentiles. Grey dots indicate the mean proportion of overlap. Each value was calculated from model posterior distributions (see Materials and Methods for details).

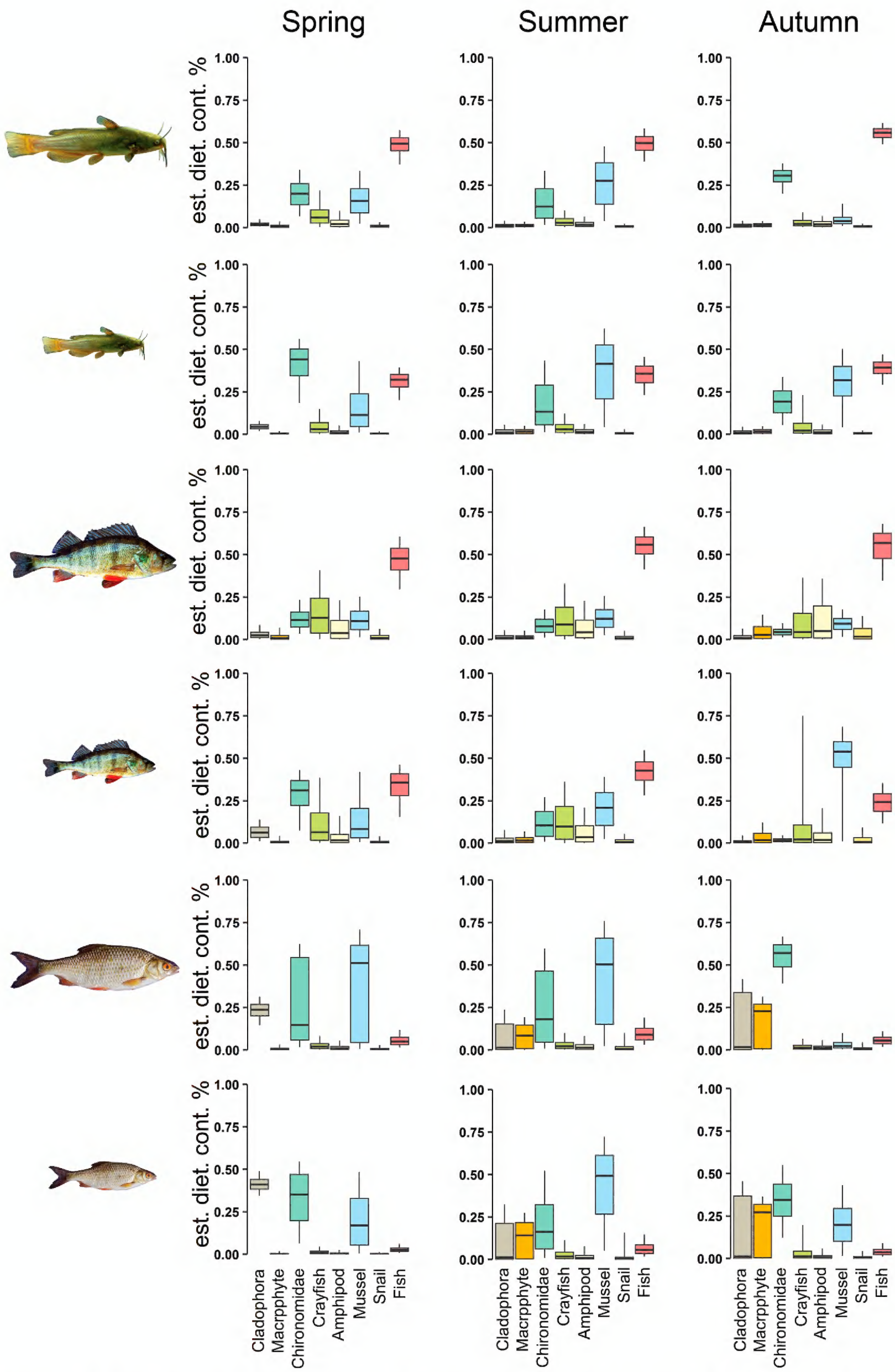

Figure 3. Estimated seasonal contributions of food sources to the diet of large and small size groups of black bullhead (*Ameiurus melas*), perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) based on Bayesian stable isotope mixing models. Fish were collected in Lake Balaton (Hungary) in spring (from 26 May to 28 May), summer (from 15 August to 25 August) and autumn (from 29 October to 3 November) of 2022. Axes show different food sources and their estimated contributions to the diet. Whiskers indicate 95% credible intervals. Boxplots indicate 25th, 50th and 75th percentiles. Each value was calculated from model posterior distributions (see Materials and methods for details).

consumed macro invertebrates; diet of black bullhead contained mainly chironomid larvae and mussels, while the proportion of different macro invertebrate components distributed more equally in the diet of perch. Diet of small individuals of both species included both macro invertebrates and fish as main dietary components. Similarly to large conspecifics, small black bullhead fed mainly on mussels and chironomid larvae, while small perch fed on various macro invertebrates throughout the year. Proportion of fish in the diet of large black bullhead was significantly higher than in the diet of small black bullhead in each season (>0.95 probability), while there were no significant differences between seasons (spring vs. summer: probability = 0.56; spring vs. autumn: probability = 0.85; summer vs. autumn: probability = 0.77). Roach diet comprised both plant-based components and macro invertebrates in both size groups. The main macro invertebrates consumed by roach were chironomid larvae and mussel in each season. Regarding plants, only cladophora was consumed by roach in spring, while in summer and autumn, when developed aquatic macrophytes became abundant in the lake, roach shifted its plant-based diet to this food item. Although each studied fish species relied predominantly on the pelagic production in each season (77–95%, Appendix 3), the two source-one biotracer ($\delta^{13}C$) Bayesian mixing models indicated some differences in the use of primary carbon sources between black bullhead and the studied native fishes. Specifically, black bullhead utilized pelagic-based food web at a higher rate than native species independently of size group and season.

Large perch occurred in the highest trophic position in each season followed by large black bullhead (Fig. 4), but these differences were not significant (spring: probability = 0.62, summer: probability = 0.83, autumn: probability = 0.85).

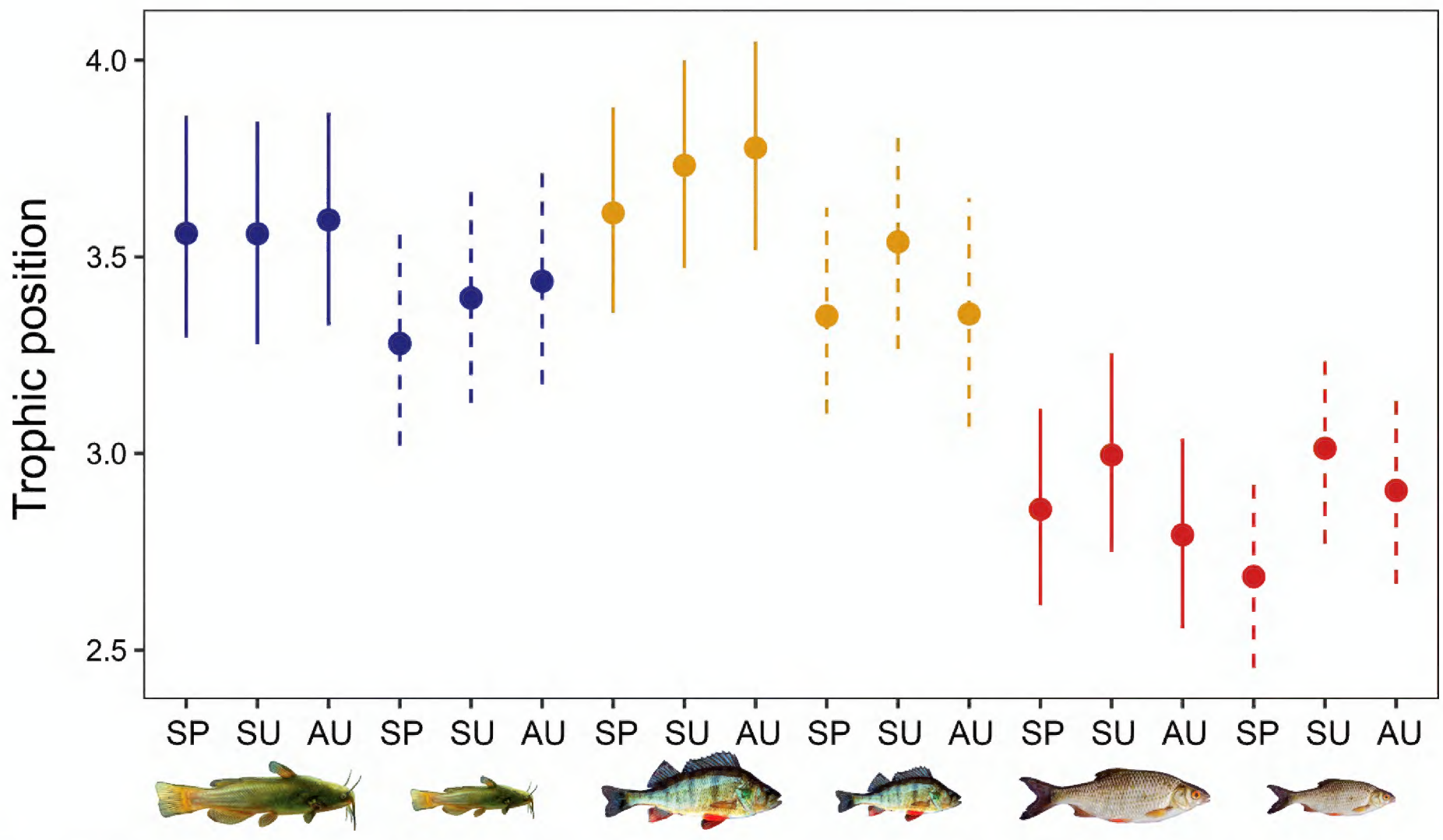

Figure 4. Estimated posterior trophic position of large and small size groups of black bullhead (*Ameiurus melas*), perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) collected in Lake Balaton in spring (SP, from 26 May to 28 May), summer (SU, from 15 August to 25 August) and autumn (AU, from 29 October to 3 November). Axes show seasons and the trophic position of species in different size groups. Each value was calculated from model posterior distributions (see Materials and Methods for details).

Similarly, trophic position of small black bullhead and small perch did not differ significantly (spring: probability = 0.66, summer: probability = 0.79, autumn: probability = 0.68), while large perch had a significantly higher trophic position (>0.95 probability) than small black bullhead and small perch in each season and in autumn, respectively. Both species had a significantly higher trophic position than roach in each season and size group.

Discussion

The most prominent impact of invasive black bullhead on native fish and ecosystems is that it can act as a predator and a competitor of food resources simultaneously (Leunda et al. 2008; Ruiz-Navarro et al. 2015; Jaćimović et al. 2021). In this study we found that black bullhead in Lake Balaton could be characterised by invertivore-piscivorous feeding habits with a substantial rate of fish consumption. Large black bullhead consumed fish in a higher proportion than small ones and the rate of fish consumption was independent of the season, confirming our predictions. However, contrary to our hypothesis, substantial food niche partitioning occurred among black bullhead and native fish species suggesting that they assimilate distinct energy sources in different proportions and thus occupy different trophic positions in the food web. In sum, two (iii and iv) out of our four predictions (see Introduction for details) were supported, while two (i and ii) were not supported by our results.

Niche partitioning facilitates the local coexistence of native and alien species that divide available resources by separating along one or more niche dimensions (e.g., diet, habitat, activity time) (Schoener 1974). According to our results, core niche segregation of black bullhead and native fishes occurred along both the $\delta^{13}C$ and $\delta^{15}N$ axes indicating differences in the diet source and that they obtain resources at different trophic levels. Black bullhead consumed mussels and chironomid larvae in a high proportion. Since these invertebrates filter various suspended materials from the whole water column (Oliver 1971; Pinder 1986; MacIsaac et al. 1995; Spooner and Vaughn 2006), their assimilations could shift black bullhead's isotopic signal towards a more negative direction along the $\delta^{13}C$ axis. In contrast, the diet of perch was more heterogeneous regarding macro invertebrates, while the diet of roach included considerable amount of plant material, which contributed to the more enriched $\delta^{13}C$ values of these species (Post 2002; Guinan Jr et al. 2015; Yu et al. 2016).

Variation in the foraging areas could also contribute to the variability of $\delta^{13}C$ values. For example, Coulter et al. (2019) highlighted that niche partitioning of native and alien planktivorous fishes might have been driven by the distinct rate of using pelagic vs. littoral/benthic food sources. Similarly, Mumby et al. (2018) emphasized the role of habitat partitioning in the very limited extent of isotopic niche overlap in an offshore fish assemblage. However, our studied species relied predominantly on the pelagic production of the lake indicating little importance of foraging migration in niche partitioning. The dominance of pelagic-based food components in the diet could be unexpected considering that the studied fishes inhabit mainly the littoral zone (Specziár, 2010; Specziár et al. 2013). However, Lake Balaton is a shallow and turbid lake, and these properties facilitate the homogenization of the materials produced in different zones of the lake. This suggests that the isotopic signature of species living in the littoral zone can be affected by food components produced in the pelagic area (Monroy et al. 2014). Apparent niche segregation of perch and black bullhead from roach along $\delta^{15}N$ axis was driven mainly by the distinct rate of pisciv-

ory. Although we found significant differences in $\delta^{15}N$ values between black bullhead and perch, too, they still occupy the same trophic position, at least within size groups. These findings highlight that although different resource-consumer dynamics exist between black bullhead and perch, they play similar functional roles in the food web.

Isotopic areas of native species overlapped only slightly or negligibly with the isotopic area of black bullhead throughout the year and in each size group suggesting relatively low potential for competition among them. It is important to note that our results do not rule out that black bullhead competes for food with other fishes in the lake. It does highlight, however, that the species' omnivorous or invertivore-piscivorous feeding habit detected in other ecosystems do not necessarily predetermine that black bullhead would compete with co-occurring native fishes from similar feeding groups (Leunda et al. 2008; Jaćimović et al. 2021). The potential lack of competition among the studied species may emerge through different mechanisms. Firstly, black bullhead probably exploits open resource niches, which may exist in anthropogenically modified and intensively utilized ecosystems like Lake Balaton (see e.g. Czeglédi et al. 2019), or obtains resources that are underused by native species (Coulter et al. 2019). Alternatively, given that the first occurrence of the black bullhead in Lake Balaton dates back to the 1980s, it is possible that the native species has already undergone competition-induced niche shifts during their co-existence. For testing these assumptions, exploring historical isotopic signatures of preserved specimens from the pre-invasion period, and using a finer taxonomic resolution of potential prey items would be necessary (e.g. complement our SIA with gut-content analyses). However, niche size of black bullhead was significantly smaller than that of roach and did not show a clear distinction with the niche size of perch, of the three studied species, black bullhead was the only one that showed significant seasonal niche size variability in both size groups. Based on these results, we assume that black bullhead does not have a permanently wide foraging niche by feeding on a variety of prey items in Lake Balaton, which can be an advantageous strategy in the invasion process (Tonella et al. 2018), but instead varies its foraging niche size temporally. Plasticity in niche size also supports the avoidance of competition with native fish and may facilitate the fast spread and wide establishment of black bullhead, similarly to other successful invaders (Almeida et al. 2012; Pettitt-Wade et al. 2015; Dominguez Almela et al. 2021).

Although black bullhead was predicted to occupy intermediate trophic position between perch and roach, the preference towards animal-based food items raised the species into the same level where the invertivore-piscivorous perch occurs. By taking into consideration all fishes in Lake Balaton, only piscivore apex predators such as pike, pikeperch (*Sander lucioperca* Linnaeus, 1758), European catfish (*Silurus glanis* Linnaeus, 1758), and asp (*Leuciscus aspius* Linnaeus, 1758) are in a higher position in the food web (Specziár, 2010). The substantial rate of fish consumption revealed by this study suggests that black bullhead is likely to have a large impact on native fish assemblage through predation. Our results thus are in accordance with Ruiz-Navarro et al. (2015), who found no isotopic niche overlap between black bullhead and roach in the invaded area and emphasized the importance of direct predation as the main adverse effect of black bullhead on native fish. Similarly to Leunda et al. (2008), who also highlighted the potential detrimental effects of black bullhead on local native fish fauna through predation, we found an ontogenetic diet shift between the species' size groups with an increasing rate of fish consumption with body size. For larger individuals, capturing protein-rich fish prey is probably more energetically profitable than choosing macro invertebrates

with hardly digestible calcium carbonate shells and chitin exoskeleton. Here, it is important to note that neither SIA nor gut content analyses allow to ascertain the origin (i.e., live or carcass) of fish preys and, in recent laboratory experiments, Preiszner et al. (2020, 2024) revealed that black bullhead may exhibit scavenging behaviour. Thus, it is possible that some of the fish consumed by black bullhead were carcasses, although carcass feeding has been suggested to be rather a complementary foraging strategy and not pivotal in their diet (Preiszner et al. 2020).

In conclusion, we compared the diet of black bullhead with an omnivorous and an invertivore-piscivorous fish species and found proof for intensive fish predation but revealed little evidence of actual feeding competition with native fish in Lake Balaton. The high proportion of fish in the diet of black bullhead might raise concerns in its invaded range. For example, in Lake Balaton, its population size suddenly increased (relative abundance of the species was <1% between 1996 and 2018 and 14.5% in 2022, unpublished standard monitoring data), and thus the species may represent a threat for native, small-sized fishes. Its predation can be especially significant during the spawning period, in spring and early summer when juvenile native fish are recruiting in the lake. Moreover, current human-induced alterations in the environmental characteristics of the lake (e.g. establishing wave-free harbours where dense submerged macrovegetation can develop) may further facilitate the spread and population growth of black bullhead (Jenkins 1957; Copp et al. 2016). In light of our compelling evidence of potential risks posed by the black bullhead, in ecosystems where its population expands, we strongly recommend urgent management actions, such as the selective removal of the species (see e.g., Jaćimović et al. 2023) to minimize its adverse impacts on the native fish fauna.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: IC, TE. Data curation: IC, AS. Formal analysis: IC. Funding acquisition: IC, TE. Investigation: IC, TE, AS, PT, BB, GB, AM, BP. Methodology: IC, BB, TE, AM, PT, AS, BP, GB. Project administration: IC. Resources: IC, TE. Supervision: IC. Visualization: IC. Writing - original draft: IC. Writing - review and editing: AS, TE, BB, BP, GB, AM, PT.

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Data availability

All of the data that support the findings of this study are available from the corresponding author upon reasonable request.

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Appendix 1

Table A1. Seasonal niche size differences within species and size groups. Values indicate probability values from posterior distributions.

Black bullhead							
	small size group				**large size group**		
	spring	summer	autumn		spring	summer	autumn
spring	NA			spring	NA		
summer	>0.95	NA		summer	0.57	NA	
autumn	>0.95	0.89	NA	autumn	>0.95	>0.95	NA
Perch							
	small size group				**large size group**		
	spring	summer	autumn		spring	summer	autumn
spring	NA			spring	NA		
summer	>0.95	NA		summer	0.69	NA	
autumn	>0.95	>0.95	NA	autumn	0.76	0.58	NA
Roach							
	small size group				**large size group**		
	spring	summer	autumn		spring	summer	autumn
spring	NA			spring	NA		
summer	0.88	NA		summer	0.58	NA	
autumn	0.62	0.80	NA	autumn	0.78	0.72	NA

Table A2. Niche size differences between species. Values indicate probability values from posterior distributions.

Spring							
	small size group				**large size group**		
	Black bullhead	Perch	Roach		Black bullhead	Perch	Roach
Black bullhead	NA			Black bullhead	NA		
Perch	>0.95	NA		Perch	>0.95	NA	
Roach	>0.95	0.91	NA	Roach	0.86	>0.95	NA
Summer							
	small size group				**large size group**		
	Black bullhead	Perch	Roach		Black bullhead	Perch	Roach
Black bullhead	NA			Black bullhead	NA		
Perch	>0.95	NA		Perch	>0.95	NA	
Roach	>0.95	>0.95	NA	Roach	0.85	>0.95	NA
Autumn							
	small size group				**large size group**		
	Black bullhead	Perch	Roach		Black bullhead	Perch	Roach
Black bullhead	NA			Black bullhead	NA		
Perch	>0.95	NA		Perch	0.66	NA	
Roach	>0.95	>0.95	NA	Roach	>0.95	>0.95	NA

Appendix 2

Table A3. Results of Hotelling T^2 test. Upper matrix indicates Hotelling T^2 values, while lower matrix indicates P values.

Spring							
		Black bullhead		Perch		Roach	
		large	small	large	small	large	small
Black bullhead	large	NA	51.10	14.74	11.08	112.16	193.76
	small	<0.0001	NA	217.41	30.84	80.72	233.43
Perch	large	0.0030	<0.0001	NA	25.93	216.15	310.86
	small	0.0105	<0.0001	0.0001	NA	60.06	167.44
Roach	large	<0.0001	<0.0001	<0.0001	<0.0001	NA	17.58
	small	<0.0001	<0.0001	<0.0001	<0.0001	0.0012	NA
Summer							
		Black bullhead		Perch		Roach	
		large	small	large	small	large	small
Black bullhead	large	NA	4.97	22.85	20.99	78.25	91.96
	small	0.108	NA	60.06	32.25	54.51	67.23
Perch	large	0.0003	<0.0001	NA	25.84	265.79	317.83
	small	0.0005	<0.0001	0.0001	NA	111.44	119.02
Roach	large	<0.0001	<0.0001	<0.0001	<0.0001	NA	4.02
	small	<0.0001	<0.0001	<0.0001	<0.0001	0.1612	NA
Autumn							
		Black bullhead		Perch		Roach	
		large	small	large	small	large	small
Black bullhead	large	NA	13.78	257.56	39.31	310.47	355.38
	small	0.0042	NA	259.66	15.57	177.34	181.49
Perch	large	<0.0001	<0.0001	NA	35.37	476.71	380.15
	small	<0.0001	0.0023	<0.0001	NA	55.03	52.4
Roach	large	<0.0001	<0.0001	<0.0001	<0.0001	NA	10.08
	small	<0.0001	<0.0001	<0.0001	<0.0001	0.0150	NA

Appendix 3

Table A4. The relative contribution of littoral and pelagic carbon sources to the diet of fish species.

Season	Species	Size group	Source of carbon	Mean	SD	2.50%	5%	25%	50%	75%	95%	97.50%
Spring	Black bullhead	large	pelagic	0.928	0.031	0.866	0.875	0.907	0.930	0.951	0.978	0.984
			littoral	0.072	0.031	0.016	0.022	0.049	0.070	0.093	0.125	0.134
		small	pelagic	0.927	0.029	0.869	0.877	0.907	0.929	0.949	0.973	0.980
			littoral	0.073	0.029	0.020	0.027	0.051	0.071	0.093	0.123	0.131
	Perch	large	pelagic	0.864	0.035	0.799	0.807	0.838	0.864	0.887	0.923	0.935
			littoral	0.136	0.035	0.065	0.077	0.113	0.136	0.162	0.193	0.201
		small	pelagic	0.861	0.030	0.803	0.811	0.840	0.861	0.882	0.910	0.920
			littoral	0.139	0.030	0.080	0.090	0.118	0.139	0.160	0.189	0.197
	Roach	large	pelagic	0.850	0.032	0.790	0.799	0.829	0.850	0.872	0.903	0.913
			littoral	0.150	0.032	0.087	0.097	0.128	0.150	0.171	0.201	0.210
		small	pelagic	0.846	0.030	0.790	0.798	0.825	0.847	0.867	0.896	0.906
			littoral	0.154	0.030	0.094	0.104	0.133	0.153	0.175	0.202	0.210
Season	**Species**	**Size group**	**Source of carbon**	**Mean**	**SD**	**2.50%**	**5%**	**25%**	**50%**	**75%**	**95%**	**97.50%**
Summer	Black bullhead	large	pelagic	0.941	0.026	0.886	0.894	0.924	0.943	0.961	0.981	0.985
			littoral	0.059	0.026	0.015	0.019	0.039	0.057	0.076	0.106	0.114
		small	pelagic	0.935	0.028	0.878	0.887	0.916	0.937	0.956	0.977	0.983
			littoral	0.065	0.028	0.017	0.023	0.044	0.063	0.084	0.113	0.122
	Perch	large	pelagic	0.869	0.031	0.810	0.818	0.847	0.869	0.891	0.921	0.930
			littoral	0.131	0.031	0.070	0.079	0.109	0.131	0.153	0.182	0.190
		small	pelagic	0.856	0.030	0.797	0.806	0.836	0.855	0.876	0.906	0.914
			littoral	0.144	0.030	0.086	0.094	0.124	0.145	0.164	0.194	0.203
	Roach	large	pelagic	0.879	0.030	0.821	0.830	0.858	0.879	0.900	0.929	0.939
			littoral	0.121	0.030	0.061	0.071	0.100	0.121	0.142	0.170	0.179
		small	pelagic	0.866	0.032	0.807	0.815	0.844	0.866	0.888	0.919	0.931
			littoral	0.134	0.032	0.069	0.081	0.112	0.134	0.156	0.185	0.193
Season	**Species**	**Size group**	**Source of carbon**	**Mean**	**SD**	**2.50%**	**5%**	**25%**	**50%**	**75%**	**95%**	**97.50%**
Autumn	Black bullhead	large	pelagic	0.945	0.022	0.899	0.906	0.931	0.946	0.961	0.977	0.981
			littoral	0.055	0.022	0.019	0.023	0.039	0.054	0.069	0.094	0.101
		small	pelagic	0.942	0.022	0.894	0.903	0.927	0.944	0.958	0.975	0.980
			littoral	0.058	0.022	0.020	0.025	0.042	0.056	0.073	0.097	0.106
	Perch	large	pelagic	0.832	0.027	0.780	0.788	0.814	0.832	0.850	0.875	0.884
			littoral	0.168	0.027	0.116	0.125	0.150	0.168	0.186	0.212	0.220
		small	pelagic	0.824	0.025	0.775	0.783	0.808	0.825	0.841	0.866	0.873
			littoral	0.176	0.025	0.127	0.134	0.159	0.175	0.192	0.217	0.225
	Roach	large	pelagic	0.776	0.025	0.727	0.735	0.760	0.776	0.792	0.816	0.824
			littoral	0.224	0.025	0.176	0.184	0.208	0.224	0.240	0.265	0.273
		small	pelagic	0.766	0.025	0.718	0.725	0.749	0.767	0.783	0.808	0.816
			littoral	0.234	0.025	0.184	0.192	0.217	0.233	0.251	0.275	0.282